Practical Analysis of Complex Coacervate Systems

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Several theoretical treatments of complex coacervation exist: the Voorn–Overbeek theory (1–3), the Veis–Aranyi "dilute phase aggregate model" (4), the Nakajima–Sato model (5), and the Tainaka model (6, 7). These theories are contradictory on many points including the roles of electrostatic and entropy forces, the significance of Huggins interactions, and the type of charge interaction. In this paper an attempt is made to resolve these contradictions and to further characterize the coacervation process. Gelatin/acacia coacervation, the practical example on which the Voorn–Overbeek theory was based, and albumin/gelatin coacervation, which should fit the Veis–Aranyi model, are systematically evaluated. The effects of pH, ionic strength, and polyion concentration are reported. Microelectrophoretic measurements were used to determine optimum pH and ionic strength requirements for complex coacervation. Complex coacervation was suppressed at both high and low ionic strength. All of the above theories predict suppression of coacervation at high ionic strength, but not at low ionic strength. The effect of ionic strength on complex coacervation differed as the total concentration of the polyions was altered. The Voorn–Overbeek theory proved inadequate to describe gelatin/acacia coacervation under the variety of conditions studied. The Veis–Aranyi model as adapted by Tainaka explained most of this data. The albumin/gelatin system followed the Veis–Aranyi model and conformed equally well with Tainaka's adaptation of this theory.

INTRODUCTION

Complex coacervation may result on mixing oppositely charged polyions in aqueous media. This is the separation of the polyion mixture into two distinct phases: a dense coacervate phase, which is relatively concentrated in the polyions, and a dilute equilibrium phase (Bungenberg de Jong (8)). Bungenberg de Jong and co-workers carried out an extensive characterization of complex coacervation between gelatin and acacia (8). Their studies indicated that complex coacervation was dependent on the molecular weights, concentration, and ratio of the two interacting polyions and on the ionic strength, pH, and temperature of the media. A random coil configuration for both macromolecules was also considered important.

A theoretical treatment of complex coacervation was proposed by Overbeek and Voorn, on the basis of the studies of Bungenberg de Jong on gelatin/acacia coacervation. Voorn (1, 3) and Overbeek and Voorn (2) explained the spontaneous coacervation which occurred between gelatin and acacia as a competition between electrical attractive forces which tend to accumulate charged polyions and entropy effects which tend to disperse them. The macromolecular skeins of two oppositely charged polyions associate together as a result of electrostatic forces to form a coacervate phase, entrapping water between their loops. The water present in the coacervate phase contributes to the entropy allowing a number of possible arrangements of the macromolecules. The coacervate is therefore liquid in nature and readily reversible.

The Voorn-Overbeek theory assumes that there is a random coil configuration of the polyions in both phases, that solvent–solute interactions are negligible, and that the elec-

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trostatic interactive forces are of a distributive nature. If the polyions were completely unfolded, rather than in the random coil configuration, it would be difficult to entrap water within the polyion skeins and coacervation would be unlikely to occur. Distributive electrostatic interactions allow for an overall electrical neutrality in the coacervate yet the molecules are free to move around in the liquid state. Site-specific interactions would tend to form either aggregates or precipitates.

In their theoretical treatment Overbeek and Voorn (2) used the Debye–Huckel equations (9, 10) for the electrical interaction term and the Flory–Huggins theory (11–15) for the entropy term. They showed quantitatively that the critical conditions for complex coacervation to occur were met for a two-component system consisting of water and a polyion salt when $\sigma^3 r \geqslant 0.53$. That is, when the charge density (σ) or the molecular weight (r) or both were sufficiently large. Voorn extended this theoretical analysis to three- and four-component systems. The critical value ($\sigma^3 r$) increases to $\geqslant 1.06$ when microions are present (three-component systems) and is increased further if more than one component is added. In a four-component system microions and excess polyions are present. By interpretation of three- and four-component systems Voorn explained the suppression of coacervation by excess salt ions and by unequal polyion ratios, both of which occur in the practical situation (1, 16, 17). Voorn surmised that microions would increase the solubility of the polyions in the equilibrium fluid and so reduce the percentage of polyions in the coacervate. The charge density is also decreased as a consequence of counterion screening.

A second theory to explain complex coacervation, the "dilute phase aggregate model," was developed by Veis and Aranyi to account for a practical case where the Voorn–Overbeek theory did not apply (4). Veis and Aranyi were able to form complex coacervates between oppositely charged gelatins where the critical conditions of $\sigma^3 r \geqslant 0.53$ were not met. In fact coacervation occurred when $\sigma^3 r$ was several orders of magnitude less than 0.53. The Veis–Aranyi model is not limited by the assumptions of the Voorn–Overbeek theory. This model considers coacervation as a two-step rather than a spontaneous process as assumed by the Voorn–Overbeek theory. First, spontaneous aggregation of the oppositely charged gelatins takes place by electrostatic interaction upon mixing, forming aggregates of low configurational entropy. These aggregates, "coacervate sols," then rearrange to form the coacervate phase. Rearrangement occurs slowly, over hours or even days, and is driven by the gain in configurational entropy which occurs on the formation of a randomly mixed, concentrated coacervate phase and dilution of the aggregate phase. Veis modified the Voorn–Overbeek model to include the Huggins parameter (χ) and replaced the Debye–Huckel-derived electrostatic term with a term which is a function of concentration and charge density and represents the electrostatic free-energy change on transfer of a polymer segment from the dilute to the concentrated phase. Experimentally Veis found that both the electrostatic term and the Huggins term varied linearly with the total mixing concentration (Ct) up to a point, where a sharp discontinuity occurred. This concentration (Ct) coincided with a critical mixing concentration, above which coacervation was not detected.

There are several major differences between these two theories. Electrostatic interaction drives coacervation according to the Voorn–Overbeek theory, whereas the Veis–Aranyi model claims that the entropy gain on rearranging electrostatically induced aggregation drives coacervation. The Voorn–Overbeek theory assumes that solvent–solute (Huggins) interactions are negligible, whereas the Veis–Aranyi theory considers these of importance. The Voorn–Overbeek theory assumes that the charge interactions are of a distributive nature, allowing the molecules freedom to move around, whereas the Veis–Aranyi theory assumes that ion-paired aggregates are formed initially. According to the Voorn–Overbeek theory complex coacervation is spontaneous

on mixing the oppositely charged polyions, while the Veis–Aranyi theory deals with a system where coacervation can take hours or even days to occur.

The two-step process described in the Veis–Aranyi theory has been confirmed for gelatin/gelatin coacervation by Burgess and Carless (17, 18). They were able to detect the presence of small aggregates by light scattering and these aggregates rearranged, with the aid of temperature reduction as a second driving force, to form a coacervate phase (18). In another publication, Singh and Burgess (19) reported that flocculation as well as coacervation occurred on mixing oppositely charged albumin and alginic acid and that this system appeared to comply with the Veis–Aranyi theory of complex coacervation. On the basis of charge density and molecular weight, albumin/alginic acid complex coacervation would have been expected to follow a pattern similar to that of gelatin/acacia coacervation. The molecular weight of albumin is of the order of that of gelatin (6.7×10^4 (20) and 4.6×10^4 (16), respectively). Alginic acid and acacia both have molecular weights of approximately 2.4×10^5 (21, 22). All four polyions carry approximately the same charge at the respective coacervation–pH values studied. Although the critical conditions of the Voorn–Overbeek theory ($\sigma^3 r \geq 0.53$) for charge density and molecular weight are met, albumin/alginic acid coacervation did not comply with this theory. The Veis–Aranyi model was shown to best describe this system since albumin/alginic acid coacervation occurred in two steps, forming aggregates first and then a coacervate phase; temperature reduction was required to achieve significant levels of coacervate; and coacervation was limited to very low polyion concentrations. The deviation of this system from the Voorn–Overbeek theory was explained on the basis that the assumptions of a random coil configuration of the molecules, negligible Huggins interactions, and distributive charge interactions were not met. This was related to the extended "rod-like" structure of alginic acid under conditions of high charge density which are necessary to bring about interaction between the molecules.

Two other theories of complex coacervation have been developed: the Nakajima–Sato model (5), which is adapted from the Voorn–Overbeek theory (1–3), and the Tainaka model (6, 7), which is adapted from the Veis theory (4, 23–25). Nakajima and Sato (5, 26) studied a truly symmetrical polyion pair, oppositely charged poly(vinyl alcohol) macromolecules of high charge densities. They modified the Voorn–Overbeek theory to include a Huggins interaction parameter. They concluded that the electrostatic term calculated according to Voorn was much too small; however, they agreed with the Voorn–Overbeek theory in that the charges should be treated as distributed uniformly in both the dilute and concentrated phases.

According to the Tainaka model the polycations and polyanions form aggregate pairs in the dilute phase as described by Veis, but without specific charge pairing. These aggregates can be symmetrical where the polyions are symmetric with respect to charge and molecular size or asymmetric where the polyions are asymmetric with respect to charge and molecular size. The dilute phase aggregates condense to form a coacervate. Thus aggregates are present in both the dilute and the coacervate phases. Tainaka used the virial coefficient expansion procedure, which Veis and Gates applied in the dilute phase, for both phases. In the coacervate phase the aggregates overlap with each other resulting in electrostatic energy gain arising from the increase in the ion density in the overlapped domain. Phase separation is driven by attractive forces between aggregates, which are stronger the higher the molecular weights and the greater the charge densities of the polyions.

The Tainaka theory adequately explains self-suppression of coacervation (suppression at high polyion concentration) as stabilization of the aggregate structure at high concentration. Suppression of coacervation at high salt concentration is explained by nonsymmetrical mixing of the polyions, as in the Veis model.

Unlike the Veis model, the Tainaka model is not restricted to low charge density, although the charge density and polyion molecular weight should fall within a critical range. At very high values of polyion charge density and molecular weight a very concentrated gel or precipitate phase forms since strong, long-range attractive forces exist between the aggregates. At very low values of polyion charge density and molecular weight, the dilute solution is stabilized due to short-range repulsive forces and coacervation does not occur. Using the Tainaka theory good correlation has been shown between calculated values for phase concentrations and experimental values for both high charge density poly(vinyl alcohol) systems and low charge density gelatin systems (6).

The contradictions between the Voorn–Overbeek and the Veis–Aranyi theories may be a consequence of their specificity; the Voorn–Overbeek theory was based on gelatin/acacia coacervation, and the Veis–Aranyi theory was based on gelatin/gelatin coacervation. The Nakajima–Sato model was also based on a specific system, high charge density oppositely charged poly(vinyl alcohol). The Tainaka model is more broadly based and has been shown to be applicable to both a high charge density system, poly(vinyl alcohol), and a low charge density system, gelatin (6).

In this study an attempt is made to find common ground between the coacervation theories and to further characterize the coacervation process. Gelatin/acacia and albumin/gelatin coacervation are investigated in depth, with particular attention given to the limiting conditions of ionic strength and polyion concentration and to the interrelationship of these two variables. These systems were selected on the basis of the charge densities, the molecular weights, and the configurations of the polyions. The gelatin/acacia system was chosen as this is the system on which the Voorn–Overbeek theory was based. The albumin/gelatin system was chosen as albumin has a molecular weight and a charge density similar to those of gelatin and should follow the Veis–Aranyi model of complex coacervation.

Various authors have studied coacervation and have investigated the effects of different parameters on coacervate yield. However, the literature is deficient in several aspects. Most authors have failed to state the exact conditions under which their studies took place, particularly with regard to microion content and the molecular weights of the polyions. Polyions such as gelatin and acacia may contribute considerable quantities of salts and these should be either removed or accounted for when calculating the ionic strength. Most investigations have not included studies at low ionic strength and at low polyion concentration.

MATERIALS AND METHODS

Two types of gelatin were obtained from Gelatin Products, Ltd., UK: Type A (acid processed) gelatin and Type B (alkali processed) gelatin. The gelatins had the following characteristics: Type A, Bloom No. 256; isoelectric pH, 8.3; $M\bar{n}$, 4.7×10^4; and ash content, 0.2% (w/w); Type B, Bloom No. 250; isoelectric pH, 4.8; $M\bar{n}$, 4.6×10^4; and ash content, 1% (w/w). The isoelectric pH values were measured by microelectrophoresis and by ion exchange. Bovine serum albumin (molecular weight, 6.7×10^4), acacia (molecular weight, 2.4×10^5), Amberlite IR-120P (cation exchanger), and Amberlite IRA-400 (anion exchanger) were obtained from Sigma Chemicals (St. Louis, MO). Colloidal silica (Minusil) of particle size 2.7 μm (geometric weight-mean diameter) was obtained from Zeta-Meter, Inc. (New York). Hydrochloric acid, sodium hydroxide, sodium chloride, and other chemicals used were of analytical grade and were obtained from Fisher Scientific, (Springfield, NJ). The polyion solutions were prepared by dispersion in distilled water at 40 $\pm$ 0.1°C. The macromolecules were allowed to hydrate completely; this took 30 min to 1 h. Following hydration the solutions were

deionized by mixing for 30 min at 40 ± 0.1°C with Amberlite resins IR-120P and IRA-400 prior to use. This method is an adaptation of the method of Janus *et al.* (27).

Microelectrophoresis

The charge carried by the polyions affects coacervation. The extent of coacervation and the optimum conditions of pH and ionic strength can be predicted from microelectrophoresis data (16). A Lazer-Zee meter, Model 501, was used in conjunction with a plexiglass cell. Microelectrophoresis was conducted at 1 m*M* NaCl unless otherwise stated. In order to maintain constant ionic strength as the pH was varied (2–10), 1 m*M* NaOH and 1 m*M* HCl solutions were used. The polyions were adsorbed onto Minusil prior to microelectrophoresis (16). A 0.02% (w/v) polyion solution and a 0.01% (w/v) Minusil suspension were used. The ζ potential was the mean of at least 20 readings.

Dry Coacervate Yield Determination

Dry coacervate yield was used to measure the extent of coacervation as the polyion concentration, ionic strength, and pH were varied. Coacervate volume may not give a true measurement of the degree of coacervation (16). Equal volumes of the deionized polyion solutions under study were mixed with constant stirring (300 rpm) at 40 ± 0.1°C for 1 h, at the appropriate polyion concentration and pH and under the appropriate ionic strength conditions. The gelatin/acacia mixtures were left to equilibrate for 4 h. The albumin/Type A gelatin mixtures required temperature reduction to aid coacervate formation. The albumin/Type A gelatin mixtures were equilibrated at 40 ± 0.1°C for 1 h, and the temperature was slowly reduced to 15 ± 0.1°C and equilibrated for 8 h to allow high coacervate yields. Following equilibration the coacervates were centrifuged (1000 to 2000 *g*) at their equilibration temperatures to ensure complete phase separation. The two phases were then separated and added microions were removed by deionization of each phase at 40°C; known volumes of water were added as necessary. The coacervate and equilibrium phases were dried at 60°C for 6 to 10 h and weighed to obtain the coacervate yield as a percentage of the total amount of polyions added.

Polyion concentration determination. Coacervate yields were determined at different total concentrations of the two polyions under investigation (1:1 mixture), maintaining constant pH and ionic strength at the optimum values for maximum coacervation (as determined from microelectrophoresis data and previous studies).

pH determination. Coacervate yields were determined at different pH values (2–8), maintaining constant ionic strength and polyion concentration at the optimum values for maximum coacervation (as determined from microelectrophoresis data and previous studies).

Ionic strength determination. Coacervate yields were determined at different ionic strengths (0 to 100 m*M*), maintaining constant pH and polyion concentration at the optimum values for maximum coacervation (as determined from microelectrophoresis data and previous studies).

RESULTS AND DISCUSSION

Microelectrophoresis

The requirement that one polymer carry a positive charge and the other a negative charge restricts complex coacervation to a finite pH range. This range can be predicted from the pH–ζ potential profile of the polyions (Fig. 1) and specifically the optimum pH for maximum coacervation of a given mixture can be predicted to occur at the electrical equivalence pH (EEP) (16). This is the pH where the two polyions carry equal and opposite charges. The optimum pH values for maximum coacervation of the polyion mixtures are: Type A gelatin/acacia, pH 3.8; Type B gelatin/acacia,

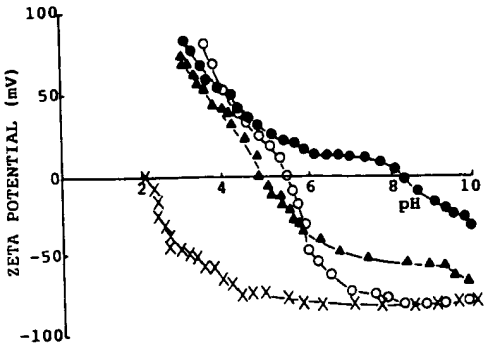


FIG. 1. The effect of pH on the ζ potential of Type A gelatin, Type B gelatin, albumin, and acacia (ionic strength, 1 mM). (●) Type A gelatin; (▲) Type B gelatin; (○) albumin; (×) acacia.

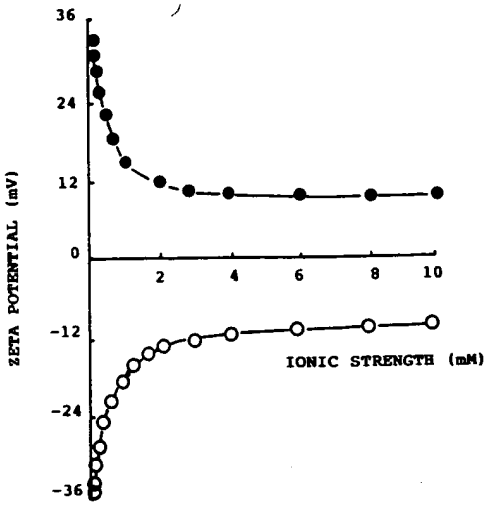


FIG. 3. The effect of ionic strength on the ζ potential of Type A gelatin and albumin. (●) Type A gelatin; (○) albumin.

pH 3.6; and albumin/Type A gelatin, pH 5.7. From Fig. 1 it can also be determined that coacervation of Type A gelatin/acacia is likely to occur over a wider pH range than Type B gelatin/acacia and that the charge density under optimized pH conditions is much lower for the albumin/Type A gelatin system than for either of the gelatin/acacia systems.

The ionic strength–ζ potential profiles may also be used to predict coacervation (16). The ionic strength of the media affects the charge carried by polyions through the screening effect of the counterions. The effects of ionic strength on the ζ potential of gelatin and acacia at their EEP values are shown in Fig. 2 and the ionic strength–ζ potentials of albumin and Type A gelatin at their EEP value are shown in Fig. 3. As the ionic strength is increased the charge on all four polyions decreases. There is an extremely rapid decrease in charge between ion-free conditions and an ionic strength of 5 mM.

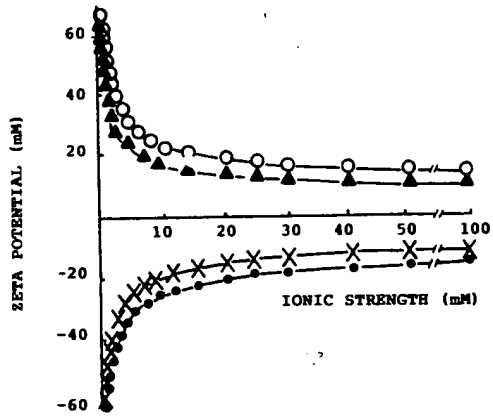


FIG. 2. The effect of ionic strength on the ζ potential of Type A gelatin, Type B gelatin, and acacia at the EEP values of Type A gelatin and acacia (pH 3.8) and Type B gelatin and acacia (pH 3.6). (○) Type A gelatin at pH 3.8; (▲) Type B gelatin at pH 3.6; (×) acacia at pH 3.6; (●) acacia at pH 3.8.

The pH–charge and ionic strength–charge profiles for albumin are very similar to those of Type B gelatin. Both gelatin/gelatin (17, 18) and albumin/Type A gelatin coacervate systems have relatively low charge densities at their EEP values, below a critical minimum value for complex coacervation according to Burgess and Carless (16) and the Voorn–Overbeek theory (3). Oppositely charged mixtures of Type A gelatin and albumin may therefore behave similarly to gelatin/gelatin mixtures and coacervate as described in the Veis–Aranyi dilute phase aggregate model (4). The relatively higher charge on the polyions in the albumin/Type A gelatin system at low ionic strength (below 5 mM) indicates that

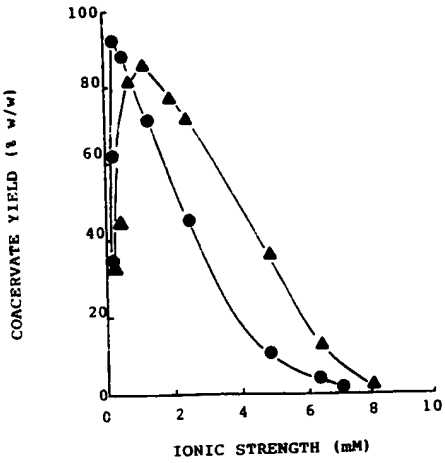


FIG. 4. The effect of ionic strength on the coacervate yield of 0.5% (w/v) gelatin/acacia mixtures. (●) Type A gelatin/acacia (pH 3.8). (▲) Type B gelatin/acacia (pH 3.6).

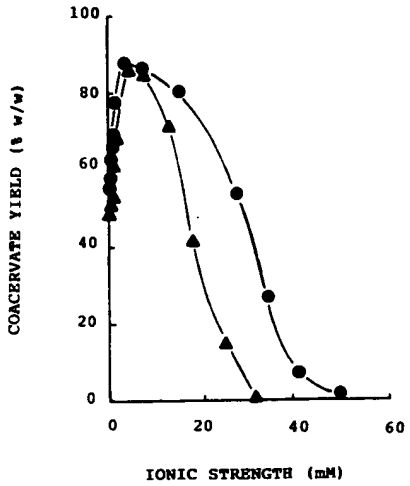


FIG. 6. The effect of ionic strength on the coacervate yield of 2.0% (w/v) gelatin/acacia mixtures. (●) Type A gelatin/acacia (pH 3.8); (▲) Type B gelatin/acacia (pH 3.6).

coacervation may proceed more readily at low ionic strength as occurs with gelatin/gelatin coacervation (17, 18).

Gelatin/Acacia Coacervation

Complex coacervates of Type A gelatin and acacia and Type B gelatin and acacia were prepared in the polyion concentration range 0.5 to 15% (w/v) and the ionic strength range 0 to 100 mM. The effects of ionic strength on the coacervate yields obtained from the various mixtures are shown in Figs. 4–7. At total polyion concentrations of 15% (w/w) and

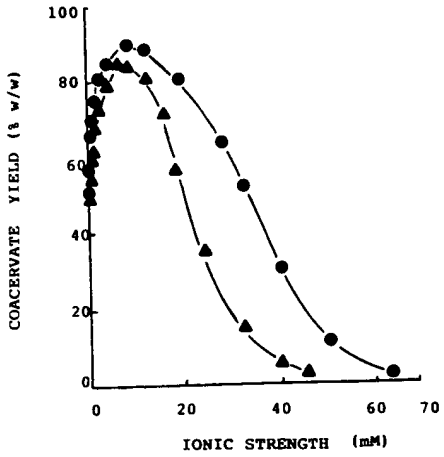


FIG. 5. The effect of ionic strength on the coacervate yield of 1.0% (w/v) gelatin/acacia mixtures. (●) Type A gelatin/acacia (pH 3.8); (▲) Type B gelatin/acacia (pH 3.6).

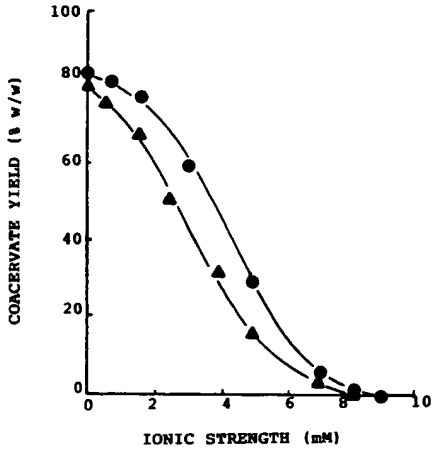


FIG. 7. The effect of ionic strength on the coacervate yield of 10.0% (w/v) mixtures of Type A gelatin/acacia and 7.0% (w/v) mixtures of Type B gelatin/acacia. (●) Type A gelatin/acacia (pH 3.8); (▲) Type B gelatin/acacia (pH 3.6).

above, coacervation was completely suppressed in both coacervate systems. Total polyion concentrations of 7% (w/v) for the Type B gelatin/acacia mixture and of 10% (w/v) for the Type A gelatin/acacia mixture were the highest concentrations at which the two mixtures formed measurable amounts of coacervate (Fig. 7). At polyion concentrations just outside the coacervation range the mixtures appeared opalescent, while at concentrations distant from the coacervation range clear solutions resulted on mixing.

The concentration of added microions tolerated varied with the total polyion mixing concentration in both systems, increasing with an increase in the polyion concentration up to a maximum at a polyion concentration of approximately 1% (w/v) (Fig. 5) and then decreasing. The microion concentration at which maximum coacervation occurs changes with polyion concentration as does the value of this maximum. Under ion-free conditions the coacervate yield for gelatin A/acacia increases from zero at a total polyion concentration of 0.5% (w/v) to 85.3% (w/w) at a total polyion concentration of 5% (w/v) and then falls again to zero at a total polyion concentration of 14% (w/v). By comparison under the optimized ionic strength conditions for complex coacervation of each mixture the percentage coacervate yield is at a maximum at the lowest polyion concentration studied (0.5% (w/v)) and decreases as the total polyion concentration is increased.

Suppression of coacervation by the addition of microions is predicted in the Voorn–Overbeek theory. Veis *et al.* (25) reported suppression of gelatin/gelatin complex coacervation by the addition of microions, and both the Tainaka and the Nakajima–Sato models predict suppresion of coacervation by the addition of microions. However, suppression of coacervation on reducing the salt concentration is not predicted in any of the above models. Most authors have not worked at very low ionic strengths and only Burgess and Carless (17) and Singh and Burgess (19) have reported this effect. Although Veis and co-workers (4, 23–25) did perform their investigations under ion-free conditions they did not include studies on the effect of added ions on their system.

The variation in the effect of added microions on the coacervation of different polyion mixing concentrations may be a result of the degree of entanglement (intermolecular interaction) between the polyions in solution. At low total polyion concentrations (0.5% (w/v) and below) and at temperatures of 35°C and above, gelatin molecules are individually dispersed with no overlap occurring between neighboring molecules (28). On mixing such solutions of oppositely charged gelatin and acacia under ion-free conditions coacervation was not observed (Fig. 3). The charge density of both the polyions is very high at low ionic strength (Fig. 3). These highly charged molecules may be in the extended state rather than in random coils (as assumed in the Voorn–Overbeek theory) and site-specific charge interactions may occur between these polyions forming ionic paired aggregates. It is also possible that no interaction has taken place, but this is unlikely since the polyions are highly charged. The critical condition $\sigma^3 r$ may be too high for coacervation to occur, although no upper critical value of $\sigma^3 r$ is suggested in the Voorn–Overbeek theory. Sato and Nakajima (21) have reported flocculation and precipitation on mixing positively and negatively charged poly(vinyl alcohol) solutions of high charge densities. However, neither precipitation nor flocculation was observed here. The data appear to fit Tainaka's model which predicts that coacervation will not occur when the charge density and/or the polyion molecular size is too high.

The addition of microions to these low polyion concentration mixtures, up to an ionic strength of 0.075 mM in the case of Type A gelatin/acacia and 0.15 mM in the case of Type B gelatin/acacia, resulted in opalescence. Microions reduce the overall charge on the polyions and increase the coiling of their skeins; however, at the microion concentration

levels stated above the shape and/or charge density of the molecules must still be unfavorable for coacervation. Fitting these data to Veis' theory the electrostatic energy which would be lost on the breakup of these aggregates must be in excess of the energy gain which would result from the increase in entropy on formation of a random coacervate phase. These data can also be applied to the Tainaka model; the long-range attractive forces between the aggregates must be insufficient to overcome the short-range repulsive forces and bring about coacervation. On further increase in ionic strength (0.125 mM, Type A gelatin/acacia; and 0.25 mM, Type B gelatin/acacia) coacervation occurs, the conditions now being energetically favorable for coacervation. At the ionic strengths where coacervation is at a maximum (4 mM, Type A gelatin/acacia; and 9 mM, Type B gelatin/acacia) the overall charge and degree of coiling of the polyions must be optimum for coacervation. At high ionic strength coacervation is suppressed as a result of the salt ions forming a dense atmosphere around the polyions and then preventing electrostatic interaction.

At total polyion concentrations above 0.5% (w/v) neighboring molecules will overlap, particularly at low ionic strength, where the molecules are highly charged and therefore likely to be present in the extended state. On mixing two such oppositely charged gelatin and acacia solutions, the overlapping segments of the oppositely charged polyions may build up large neutralized aggregate networks entrapping sufficient water to enable rearrangement into a coacervate phase, as in Veis' model, or condensation into a coacervate phase, as in Tainaka's model. At intermediate total polyion concentrations (1% (w/v), 2% (w/v)) where the percentage of polyions in the coacervate is not at a maximum under ion-free conditions some small aggregates, which remain in the equilibrium phase, may also be formed. The proportion of these small aggregates or "coacervate sols" will diminish as the polyion concentration is increased. The addition of small amounts of microions should optimize coacervation of these systems by promoting coiling of the molecules and reducing the tendency to form coacervate sols.

At high total polyion concentrations the polyions will be interacting to such an extent under ion-free conditions that the addition of salt will only serve to suppress coacervation (Fig. 7). With further increase in the total polyion concentration, the concentration of the mixture approaches that normally found in a coacervate (10–20% (w/v)) and as a result the energy gain on formation of a coacervate is greatly reduced and eventually coacervation will be completely suppressed. Tainaka describes this phenomena as the stabilizing of the aggregates in a one-phase system. At high total polyion concentration the addition of small quantities of salt will cause a degree of coiling of the polyions and may result in sufficient disentanglement to make coacervation favorable. For example, a coacervate yield of 2.1% (w/w) was obtained on increasing the ionic strength of a 14.5% (w/v) total polyion mixture of gelatin/acacia from 0.0 to 1.0 mM. Further addition of salt, however, stabilizes the one-phase system.

It is difficult to distinguish the effects of polyion concentration and salt concentration on coacervation, as each is greatly influenced by the other. Considering both effects, a total polyion concentration of approximately 1% (w/v) and an ionic strength of around 5 mM may be taken as optimum for both gelatin/acacia systems. The systems are most stable to added salt at 1% (w/v) and high coacervate yields are obtained at this concentration.

Differences in the two types of gelatin. Although there is general agreement between the coacervate yields of equivalent gelatin A/acacia and gelatin B/acacia systems slight differences do occur which may be attributed to differences in the two gelatin molecules. Type B gelatin molecules have more extended shapes compared to Type A gelatin molecules of similar molecular weight (29). It follows that a higher ionic strength would be required

to produce a similar degree of coiling in Type B gelatin molecules than in Type A gelatin molecules, which explains the higher salt concentration necessary to induce coacervation in Type B gelatin/acacia mixtures. There will also be a greater degree of intermolecular entanglement in a Type B gelatin solution than in an equivalent Type A gelatin solution, which explains why Type B gelatin/acacia coacervation is suppressed at lower total mixing concentrations than Type A gelatin/acacia coacervation.

Albumin/Gelatin Complex Coacervation

At the EEP values of albumin and Type A gelatin these two polyions have relatively low charge densities (Fig. 3) even at an ionic strength of 1 mM. Therefore the method of Burgess and Carless (17, 18) for the production of gelatin/gelatin coacervates was applied to this system. Coacervates were prepared under ion-free conditions so that the charge densities were as high as possible, and temperature reduction was used to enhance the coacervate yield. Ion-free mixtures at the EEP, pH 5.7, were prepared at 40°C, allowed to cool to 15°C, and held at this temperature for 1 to 24 h. As shown in Fig. 8 the coacervate yield increased with time up to approximately 6 h and then plateaued. A similar trend occurred for gelatin/gelatin coacervation (17, 18). A 6 h equilibration period was selected as optimal to maximize coacervate yield for subsequent experiments. Alteration of the pH on either side of pH 5.7 resulted in extremely low coacervate yields. This effect is due not only to the inequality in the charge carried by the polyions as the pH is altered away from the EEP, but also to the reduction in the charge density of both polyions caused by the addition of microions (Fig. 3). At pH 5.6 a coacervate yield of 21% (w/w) was detected and at pH 5.8 only 14% (w/w) was detected compared to 86% (w/w) at pH 5.7. The effect of change in ionic strength on albumin/gelatin coacervate yield is shown in Fig. 9 for 1 and 2% albumin/gelatin mixtures. Albumin/gelatin coacervation is suppressed at ionic strengths above 5 mM for the 1% mixture and above 3 mM for the 2% mixture. Under ionic strength conditions just outside the coacervation range opalescence was observed while under much higher ionic strength conditions the mixtures were clear. The opalescence observed can be explained by the presence of coacervate sols as

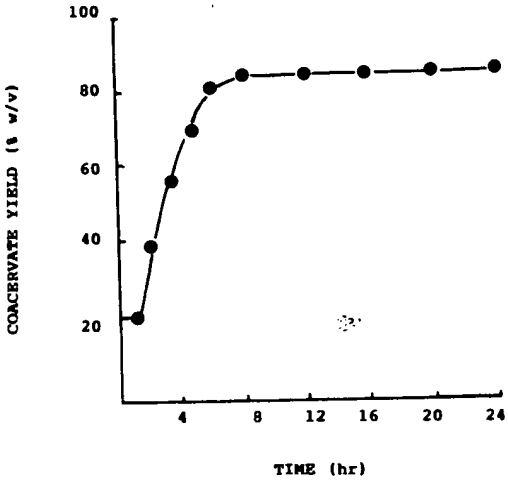


FIG. 8. The effect of time on the coacervate yield of ion-free albumin/gelatin mixtures at pH 5.7.

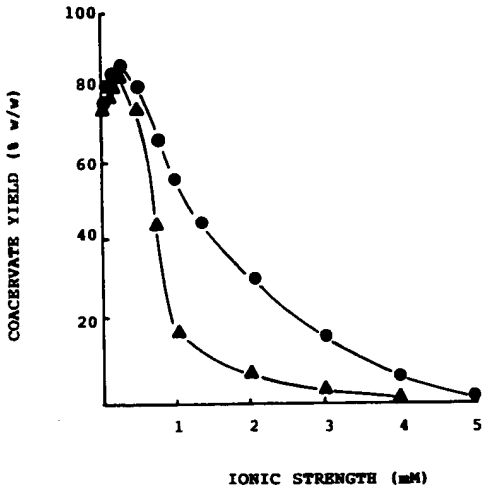
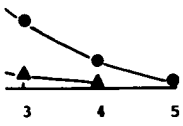


FIG. 9. The effect of ionic strength on the coacervate yield of albumin/gelatin mixtures at pH 5.7. (●) 1.0% (w/v); (▲) 2.0% (w/v).



gth on the coacervate
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described in the previous section and by Burgess and Carless (18). A slight decrease in coacervate yield is observed at low ionic strength, with maximum coacervate yield occurring at 0.3 m*M*. The general trend of coacervate yield increasing with an increase in ionic strength up to a maximum and then decreasing with a further increase in ionic strength is consistent with the data obtained here for gelatin/acacia coacervation and with previous data on albumin/alginic acid coacervation (19) and gelatin/gelatin coacervation (17, 18). This trend may be similarly explained as a consequence of the effect of added microions on the extent of coiling of the polyions and on their charge densities. The concentration of microions tolerated by albumin/gelatin coacervates is much less than that tolerated by gelatin/acacia coacervates. This is probably a consequence of the significantly lower initial (ion-free) charge density of the polyions in the albumin/gelatin system. Albumin/gelatin coacervation appears to fit the Veis–Aranyi model of complex coacervation.

Application to the Theories

The Voorn–Overbeek theory of complex coacervation was originally based on gelatin/acacia coacervation; however, the theory does not hold for this system under all practical conditions, only under conditions which are highly favorable for coacervation. In particular, the assumptions that the polyions are in a random coil state in both phases, that solvent–solute interactions are negligible, and that the interactive forces are of a distributive nature must be set aside to accommodate all possible conditions. The opalescence observed under certain conditions suggests that small aggregates have formed and that the Veis–Aranyi model or the Tainaka adaptation of this model may be more appropriate under these conditions than the Voorn–Overbeek model. The interrelationship of ionic strength and total polyion concentration indicates that Huggins interactions are significant, which is in disagreement with the Voorn–Overbeek theory. This is particularly apparent at high polyion concentrations, where both solvent–solute and solute–solute interactions are so high that there is no incentive to coacervate.

Albumin/gelatin coacervation followed the Veis–Aranyi dilute phase aggregate model, forming coacervates in a manner similar to that of the oppositely charged gelatin system. The charge densities of both polyions are low at the pH values where they carry opposite charges. The molecular weights of both these polyions are also low, 4.6×10^4 and 6.7×10^4, for gelatin and albumin, respectively. Thus on two accounts, charge density and combined molecular weight, this system does not comply with the Voorn–Overbeek model.

Suppression of coacervation at low microion concentrations occurs in both the gelatin/acacia and albumin/acacia systems and is considered to be a consequence of the high charge density of the polyions under these conditions, resulting in extended molecular skeins which interact to form small aggregates. This effect is not predicted in any of the theories.

CONCLUSIONS

Considering the two systems presented in this study, gelatin/acacia and albumin/gelatin, and two other systems previously reported, gelatin/gelatin (17, 18) and albumin/alginic acid (19), it is observed that spontaneous coacervation as described by Voorn and Overbeek on mixing oppositely charged polyions will occur only under specific conditions. The limiting conditions are the average molecular weight of the polyions and their charge densities must fall within a specific range, the polyions should be in a random coil configuration, Huggins interactions should be negligible, and the charge interaction between the molecules should be distributive in nature. When deviation from these conditions occurs, coacervation may still take place, as described by Veis and Aranyi and by Tainaka.

The gelatin/acacia system was consistent with the Voorn–Overbeek model unless the charge density was either too low or too high. At low charge density, coacervates could still be formed according to the Veis–Aranyi model. At high charge density the molecules may assume an extended rod-like configuration and thus may not fit the configurational restrictions of the Voorn–Overbeek model. The albumin/gelatin system does not comply with the charge density and molecular weight requirements of the Voorn–Overbeek model; however, coacervates do form as described in the Veis–Aranyi and Tainaka models.

None of the theories adequately describes all cases of complex coacervation. The Veis–Aranyi model could be extended by allowing the time required for rearrangement to the coacervate phase to be infinitesimally small. Oppositely charged molecules which have sufficiently high charge density could aggregate by electrostatic charge interaction and then spontaneously rearrange into a coacervate phase. This would be a partial extension of the Veis–Aranyi model to include aspects of the Tainaka model. Although the Tainaka model is not all inclusive, for example, it does not explain the reduction in coacervation at low ionic strength, it is not as restrictive as the other theories and appears to be the best general theory.

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